 MRID# 00159706

Shaughnessy #128921
Trout Acute

DATA EVALUATION RECORD

1. Chemical: Dicyandiamide

2. Test Material: Dicyandiamide; designated as SKW 8510 NS, technical grade; 100% purity. Supplied by Hazleton Laboratories America, Inc. Lot No. 84E-227 used for range-finding; lot no. 85E-022 (LH21,671A) used for definitive testing.

3. Test Type: Acute toxicity to Rainbow Trout

4. Study ID: The acute toxicity of Dicyandiamide to the Rainbow Trout, Salmo gairdneri, in a static test system. Biospherrics Project No. 84E-227RT. For: SKW Trostberg Aktiengesellschaft, Trostberg, Germany. By: Biospherics Incorporated, 4928 Wyaconda Road, Rockville, MD 20852. February 1985.

5. Reviewed by: Zigfridas Vaituzis
Microbiologist
EEB/HED
Signature: Z. Vaituzis
Date: 11/19/86

6. Approved by: Ray Matheny
Head, Section I
EEB/HED
Signature: Ray Matheny
Date: 2-13-87

7. Conclusions:

The LC_{50} in the acute toxicity to rainbow trout study as presented is not accurate because the test chemical was not in complete solution at the start of the test. The LC_{50} range, however, is considerably greater than 100 ppm. Dicyandiamide is, therefore, practically nontoxic to rainbow trout.

The study fulfills the Guidelines requirement for a rainbow trout acute toxicity study.

8. Recommendations:

N/A.

9. Background:

N/A.

10. Discussion of Individual Tests:

N/A.

11. Methods and Materials:

a. Test Organisms: Rainbow trout, Salmo gairdneri

Age/Stage of Maturity: Approximately 4 months old

Size: 39.3 mm (range 36.1 mm - 43.7 mm), n = 10

Body Weights: 1.06 g (range 0.76 g - 1.49 g), n = 10

Source: Trout Lodge, McMillin, Washington

b. Dosage Form:

Solvents/Vehicles: None

Route of Administration: Test material dissolved in water.

c. Referenced Protocol: Committee on Methods for Toxicity Tests with Aquatic Organisms. (1975) Methods for Acute toxicity tests wth Fish, Macroinvertebrates and Amphibians. EPA - 660/3-75-009.

Test Levels: Nominal concentrations in ppm: 1296; 2160, 3600, and 6000.

Dose Spacing Factor: 0.6 of next higher concentration

Number per level: 10 fish at random per container at each dilution

Holding/Acclimation: Two weeks; no mortalities

Feeding: Taken off feed 48 hours before use, and were not fed for the duration of the study.

Physical Condition: Judged acceptable for testing.

Test Conditions:

Temperature: 13 °C (constant)

Total Hardness: 115 ppm as $CaCO_3$

Dissolved Oxygen: 6.8 to 10.4 ppm; measured every 24 hours.

pH: 7.4 to 8.0; measured every 24 hours

Souce of Dilution Water: 400' deep well at Biospherics laboratory

Test Vessels: 19 liter glass carboys, containing 15 L dilution water, 28 cm deep

Static Test: 96 hours

Loading: Approximately 0.71 g/L

Aeration: Aerated until start of study

Photoperiod: 16 hr light/8 hr dark

Diet Preparation: Trout chow. No feeding during test period. Test chemical concentrations were prepared by adding known weights at dicyandiamide to the dilution water in each carboy. Preparation procedures for the test chemical concentrations were not given.

Controls: 100 percent well water used as a dilution water control.

Measured Test Levels: The client-approved protocol did not require that Biospherics analyze test solutions. Reported results are based on nominal concentrations of test material.

Observation Period: 96-hour exposure period, observations recorded every 24 hours.

Statistical Methods: The LC_{50} for 72 and 96 hr exposure levels and the 95 percent confidence limits were calculated by the moving average method (Stephan, 1979, EPA, Duluth, MN).

12. Reported Results:

Effects Criteria: Mortalities and observable abnormal behavior.

LC_{50}: 72 hr = 8500 ppm; 96 hr = 7700 ppm

NEL: 3600 ppm (96 hr)

Dose Response Data: Adequately reported.

13. Study Author's Conclusions/Quality Assurance Measures:

The 96-hr LC_{50} at dicyandiamide to the rainbow trout (Salmo gairdneri) was 7746 ppm based on nominal concentrations of test material (95% confidence limits: 6600 and 9000 ppm).

A signed and dated quality assurance statement is attached to the study.

14. Reviewer's Discussion and Interpretation of the Study:

a. Test Procedure: The following procedures are inconsistent with the HED Standard Evaluation Procedure (Acute Toxicity Test for Freshwater Fish):

a. The number of test fish used in the range-finding study is not given.

b. Total water hardness as $CaCO_3$ was 115 ppm whereas the recommended hardness is 40 to 48 mg/L as $CaCO_3$.

c. The test chemical concentration preparation procedures are not given.

b. StatisticalAnalysis: The presented data were reanalyzed and the LC_{50} was determined using a computerized version of Stephan's Binomial Probability, moving averages and probit methods. The results are in agreement with those presented by the registrant. A printout of the study is attached to this DER.

c. Discussion/Results: The reported NEL (3600 ppm at 96 hrs) and LC_{50} (7700 ppm at 96 hrs) are in actuality lower.

According to the presented data the lowest concentration showing no effect is 1296 ppm, because the 2160 ppm concentration vessel had a 10 percent mortality rate. Registrant's choice of 3600 ppm as the NEL is invalid in spite of the fact that no toxic effects were recorded at this concentration.

The LC_{50} at 96 hours as given does not represent a 96 hr exposure because all concentrations showed undissolved chemical during the first 24 hours of the test. In the 10,000 ppm concentration vessel the test material is reported to have gone into solution at 48 hours. Although an accurate LC_{50} cannot be obtained from the presented data, the range into which the LC_{50} falls is obviously well above the 100 ppm above which an accurate LC_{50} need not be determined according to the HED SEP's.

The presented study shows that dicyandiamide is practically nontoxic to rainbow trout.

d. Adequacy of Test:

1. Validation Category: Core for technical grade dicyandiamide.

2. Rationale: Fulfills Guideline requirements that the LC_{50} and NEL be > 100 ppm.

3. Reparability: N/A

15. Completion of One-Liner for Test: November 19, 1986.

16. CBI Appendix: N/A.

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10000	10	9	90	1.074219
6000	10	1	10	1.074219
3600	10	0	0	9.765625E-02
2160	10	1	10	1.074219
1296	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 6000 AND 10000 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 7745.967

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.1677541	7745.967	6446.936	10316.09

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
13	4.19971	4.637182	3.028154E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.352139
95 PERCENT CONFIDENCE LIMITS =-4.566783 AND 13.27106

LC50 = 7466.511
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 3813.357
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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